DIETARY FAT AS A PROMOTER OF LUNG CARCINOGENESIS IN THE A/J MOUSE MODEL

A PROPOSAL

Daniel W. Sepkovic, Ph.D. and Nancy J. Halley, Ph.D.

Division of Nutrition and Endocrinology

American Health Foundation

One Dana Road

Valhalla, NY USA 10595

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Daniel W. Sepkovic, Ph.D.
Research Scientist
Division of Nutrition
and Endocrinology

Nancy J. Haley, Ph.D.
Associate Chief
Division of Nutrition
and Endocrinology

ABSTRACT

In humans, smoking cigarettes has been attributed to be the most important causative factor for lung cancer. This is probably the main reason why so little attention has been given to the role of diet in the etiology of this cancer. In epidemiological studies and animal models, dietary fat has been shown to modify the onset and progression of a wide range of cancers. We propose to use the A/J mouse animal model to examine the effect of high and low dietary fat intake on lung tumor development. This model is unique in that spontaneous lung nodules begin to develop in the lung at 3-4 months of age and tumor development continues steadily to reach 100 percent at 24 months. We will use this model to compare the development and progression of lung tumors in mice given diets of different fat, but equal energy composition. We also propose to accelerate the progression of lung tumors by administering 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) to A/J mice fed various fat content diets and compare the relative rates of tumor development in this model. The goal of this project is to determine whether dietary fat intake can play a role in the incidence of tumor development in the lung. By varying the fat composition in the diet of this animal model, we hope to modify lung tumorigenesis.

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A. SPECIFIC AIMS

Dietary fat intake may play a significant role in the initiation or progression of lung cancer. Some epidemiologic studies have demonstrated that dietary fat was correlated with a significant increase in lung cancer incidence even after adjusting for smoking status. Several animal studies support the hypothesis that dietary fat intake increases the incidence of carcinoma. In one specific study, a significant increase in respiratory tract tumors was observed in hamsters fed a 20% unsaturated fat diet.

We propose to explore the association of dietary fat with lung cancer incidence using an animal model that spontaneously develops lung nodules at 3-4 months with tumor development continuing steadily to 100% in 24 months. The A/J mouse model is also sensitive to the induction of tumors by exogenous carcinogenic compounds. In preliminary studies, this animal model was established as a rapid and useful bioassay for identification of compounds which can modify NNK-induced lung tumorigenesis. The assay gives readily quantifiable results and is relatively inexpensive due to the short time span needed to give maximum tumor induction. The results of studies using the A/J mouse model compare favorably with the results obtained from similar studies using F-344 rats.

Our overall goal will be to use the A/J mouse animal model to examine the effect of dietary fat intake on lung tumor development. We will compare the spontaneous development of lung tumors in mice on different fat content diets. We will also significantly accelerate lung tumor progression by administrating NNK to mice fed different fat content diets and compare the relative rates of tumor development in this animal model.

Specifically, we propose:

1. To determine whether dietary fat intake can play a role in the promotion of lung carcinomas in the A/J mouse animal model.

2. To investigate whether high fat intake might be a modifying factor in the onset and progression of lung tumors after a single dose of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).

3. To confirm lipid changes in plasma and fatty acid composition in lung tissue as a function of dietary fat intake and relate these changes to lung tumor development.

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B. SIGNIFICANCE

While most research has focused on the role of cigarette smoking and lung cancer, only a fraction of smokers develop the disease indicating the possible role of other factors, including diet in carcinogenesis. Several studies have shown that dietary fat intake alters the incidence and time to tumor appearance of spontaneous and induced tumors of various types. In animal models, dietary fat has been shown to modify the development of pancreas tumors (1,2), mammary gland tumors (3,4,5), skin tumors (6), colon cancer (7) and liver tumors (8). Little attention has been devoted to the role of dietary fat in the etiology of lung cancer. Yet, several studies have revealed that high fat intake might be a modifying factor in the onset and progression of lung cancer. An important early study conducted by Keys et al. correlated high fat intake with a four-fold increase in lung cancer incidence in the Netherlands when compared to lung cancer incidence in Japan (9). Interestingly, the percentage of smokers in both countries was approximately equal, but fat intake in the Netherlands was four times higher than in Japan.

Byers et al. compared the diets of 450 lung cancer cases with those of 902 controls in the Western New York State Diet Study (10). Case-control comparisons were made for dietary fat, protein, fiber, calories, cholesterol, and vitamin A, C and E according to quartiles of intake and adjusting for age and pack years of cigarettes. In this study, lung cancer risk was lower for males in the lowest quartile of total dietary fat intake compared to those in the highest quartile. However, the overall trend in the association with dietary fat was not significant.

Most attention has been focused on the use of cigarettes as the major attributable cause of lung cancer in the United States. Other risk factors that could play a crucial role in the initiation of lung cancer have not been fully explored. In a case-control report of adenocarcinoma (ADC) and squamous cell carcinoma (SCC) among white females in Los Angeles county several risk factors including smoking status were evaluated (11). While SCC incidence was explained in this study almost entirely by cigarette smoking, only about 50% of ADC etiology was attributed to this risk factor. Other risk factors including childhood lung disease and early exposure to coal fires might explain another 20% of ADC cases. The remainder of cases may possibly be explained by dietary factors including Vitamin A or β-carotene and dietary fat consumption.

Animal studies strengthen the hypothesis that dietary fat may play a role in the onset of lung carcinoma. An early, though somewhat equivocal study conducted by Szepsenwol showed that the number of spontaneous lung tumors in mice was increased after feeding with egg lipids (12). A more recent study by Beems and van Beck indicated that dietary fat has a modifying effect on benzo(a)pyrene induced respiratory tract tumors in hamsters (13). The differences in tumor response were mainly due to increased

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incidences of epidermoid papillomas, epidermoid carcinomas and combined epidemoid and adenocarcinomas in the trachea and bronchii. These workers also observed that preneoplastic respiratory lesions were influenced by dietary fat.

A procedural limitation of this study was that the hamsters were instilled intratracheally with the carcinogen at weekly intervals for 15 weeks. We propose to use an animal model that does not require the administration of a carcinogen to develop lung tumors. The A/J inbred mouse strain will spontaneously develop lung nodules as young as 3-4 months and tumor development will rise steadily to 100% in about 24 months (14). The lungs of these mice are susceptible to the development of spontaneous lung tumors. They are also exquisitely sensitive to the induction of tumors by exogenous carcinogenic compounds (15-17). We will take advantage of both the spontaneity of tumor development and the sensitivity of tumor induction by carcinogens in our experimental design utilizing manipulation of dietary fat and induction by tobacco specific carcinogens.

Nicotine per se, is not generally considered to be a carcinogen (18,19). The principle stable urinary metabolites of nicotine, cotinine and both cis- and trans-nicotine-N'-oxide were examined for carcinogenic activity (20). While an early study reported the induction of lymphomas by cotinine, they were not confirmed by later studies at twice the dose level (21,22). Contemporary studies have shown that these metabolites are neither carcinogenic nor were they promoters of urinary bladder tumors in rats pretreated with the bladder carcinogen, N-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide (FANFT) (20).

The tobacco specific nitrosamines are formed from nicotine during curing and processing and during cigarette smoking. The metabolic conversion of these compounds to methylating agents provides a pathway by which the N-methyl group of nicotine can methylate DNA, a reaction that causes the impairment and alteration of the structural integrity of the cells heritable material (18,23). The possibility of an interaction between nicotine and the amount of fat consumed may accelerate or inhibit the metabolic conversion of these compounds and thus, affect tumor development. Changes in dietary lipids have been shown to affect the hepatic mixed function oxidase system (MFO) by altering the metabolism of drugs (24-26). Cytochrome P-450 and several mixed function oxidase activities are decreased in animals on fat-free or saturated fat diets and increased in animals fed polyunsaturated oils (27-31). These same MFOs are responsible for the metabolic activation of nicotine derived N-nitrosamines. An increase or decrease in the specific activity due to dietary fat intake could enhance or inhibit the carcinogenicity of these compounds.

In summary, our overall goal will be to use the A/J mouse animal model to examine the effect of dietary fat on lung tumorigenesis. We will compare the spontaneous development of lung tumors in mice fed various fat content diets. We will then accele-

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rate lung tumor progression by single dose NNK administration (at two dose levels) and examine the effects of dietary fat on lung tumor development. We will also monitor plasma lipids as well as lung tumor tissue for fatty acid changes at different times throughout the duration of these studies and relate these changes to tumor development.

C. PRELIMINARY STUDIES

Hecht and associates have recently established the A/J mouse as an animal model for lung tumorigenesis while assessing the effects of an open formula (NIH-07) and a semisynthetic (AIN-76A) diet on lung tumor development. After a two week quarantine period, the animals were given an i.p. injection of NNK in saline of varying doses (0, 1, 2.5, 5, 10 μmol) (Figure 1). Groups of 15 mice from each dosage group were sacrificed at regular intervals beginning 3 months after NNK administration. Maximum tumor induction occurred within 3.5 months. A dose response for tumor multiplicity was observed with increasing NNK dose. In a similar experiment, the same protocol was followed except the mice were fed a NIH-07 diet throughout the duration of the study. Tumor multiplicity was significantly lower than in the mice maintained on the AIN-76A diet.

The results of this study establish a rapid and useful bioassay for identification of compounds which can modify NNK-induced lung tumorigemesis (32). The bioassay gives quantitative results and is relatively inexpensive. Studies by Morse and associates (17) have demonstrated a trend of increased inhibitory activity of arylalkyl isothiocyanates towards NNK-induced lung neoplasia in A/J mice with alkyl chain length, thus lending further support for the A/J mouse animal model.

The A/J mouse model produces results that are very similar to those observed in other animal bioassays. As in the F-344 rat, treatment of A/J mice with NNK and phenethyl isothiocyanate resulted in inhibition of lung tumor formation (17). The mouse bioassay has been used to test the carcinogenicity of other tobacco specific nitrosamines. N'-nitrosonornicotine (NNN), N'-nitrosoanabasine (NNA), and 4-(methylnitrosamino)-4'-(3-pyridyl)-butanol (NNAL) have all been tested for tumorigenicity and both NNN and NNAL produced lung tumors (15). When the F-344 rat, Sprague-Dawley rat or Syrian golden hamster were used in these bioassays, the carcinogen was given in repeated weekly doses over a twenty week period. With the A/J mouse, a single and much smaller dose produced the same results in a much shorter time.

D. EXPERIMENTAL DESIGN

D.1. Procedure

Female A/J mice of 5-6 weeks of age will be obtained from Jackson Laboratories (Bar Harbor, ME) and remain under observation in the research animal facility for two weeks in quarantine. The

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mice will be housed 10 to a cage in polyethylene cages (19" x 10½" x 8") containing hardwood shavings and filter tops. The animals will be maintained according to the revised "Guide for the Care and Use of Laboratory Animals" (33). The animal room will be controlled for temperature (24 ± 2°C) light (12h cycle) and humidity (50%). All animals will be weighed at weekly intervals.

Study #1, the animals will be divided into 3 groups (n=50) and fed 3 different dietary regimens with varying food composition (see Table 1 for description of diet composition). In the A/J mouse strain, lung nodules begin to appear spontaneously in 3 to 4 months and rise steadily to almost 100% by 24 months (14). The animals will be allowed to feed ad libitum on either a low, medium or high fat diet for 8 months when 10 will be sacrificed. Ten animals will be sacrificed at 12 and 16 months, and 20 animals sacrificed at 20 months. After each sacrifice, pulmonary adenomas will be quantitated in each group.

Study 2 will last for 20 weeks. This experiment will investigate the effects of single dose administration of a carcinogen and how lung carcinogenesis is modulated by dietary fat intake. The animals will be randomly divided into 6 groups of 50 mice. Groups 1, 2 and 3 will be given low, moderate and high fat diets, respectively. These animals will be injected with a single dose of NNK (10 µmol/animal) immediately after quarantine. Groups 4, 5 and 6 will be placed on diets identical to groups 1, 2 and 3, but these animals will receive a single injection of 5 µmol/NNK/animal. The animals will be treated as above in all other respects and will be fed either a low, medium or high fat diet. Ten animals from each group will be sacrificed at 8, 12, and 16 weeks. The remaining twenty will be sacrificed at 20 weeks. After each sacrifice, pulmonary adenomas will be quantitated.

D.2. Food Consumption Measurements

Six animals from each group will be housed separately to assess average daily food consumption. Food consumption will be measured by calculating the difference between the weight of food added to food cups and that food remaining after a 24 hour feeding period. The procedure will be repeated over 3 successive days.

D.3. The High, Medium and Low-Fat Diets

The diets in these experiments are based on the recommendations of the committee on Laboratory Animal Diets of the National Academy of Sciences (35,36) with minor modifications. Table 1 presents the composition of our proposed experimental diets.

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Table 1

— Composition of Experimental Diets

Ingredient	Low Fat	Medium Fat	High Fat
Sucrose	50.0	44.8	34.5
Corn Starch	15.0	13.5	10.3
Casein	20.0	21.1	23.5
Methionine	0.3	0.3	0.3
Corn Oil	5.0	10.0	20.0
Cellulose	5.0	5.3	5.9
Minerals[a]	3.5	3.7	4.1
Vitamins[a]	1.0	1.1	1.1
Choline bitartrate	0.2	0.2	0.2
Kcal/g	3.9	4.1	4.5
% of Kcal CHO	67.5	57.3	39.6
% of Kcal protein	20.8	20.7	20.7
% of Kcal fat	11.7	22.0	39.7
	100	100	100

[a] AIN 76A mixture

As seen in Table 1, the increase in fat will be compensated for by a decrease in the amount of carbohydrate added to the diet. The high, medium and low fat diets contain 20%, 10% and 5% corn oil (w/w), respectively. Adjustments will also be made in the restricted groups to insure that despite lower overall food intake, the absolute intake of fat, vitamins and trace elements per rat will be identical in both ad libitum and restricted groups.

The three diets were designed as: low fat (10-13% of calories as fat, simulating the Japanese diet), moderate fat (20% of calories as fat), and high fat (38% of calories as fat, simulating the Western diet) (37). Based on an estimated average daily food consumption of about 45 calories, the low-fat diet will provide about 5 to 6 cal/fat per day or 12% total calories, the medium fat diet will provide 11 cal/fat per day or 22% of total calories, and the high-fat diet will provide 21 cal/fat per day or 40% of total calories as fat. Diets will be prepared by Bioserv Inc. (Frenchtown, NJ) and will be packed in 10 Kg plastic bags and stored at 4°C in the dark. The diets will be administered in powdered form and tap water will be available ad libitum.

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D.4. Necropsy and Histopathology

All animals will be sacrificed by CO_2 euthanasia. Tumors will be counted, excised, fixed in buffered formalin blocked in paraffin, sectioned and stained with eosin and hematoxylin. Histological diagnosis will be make. Prior to fixation, tumors will be weighed and measured.

D.5. Biochemical Methods

D 5.1) Fatty Acid Extraction and Analysis

Total lipids will be extracted from samples of lung tumor and adjacent lung tissue using a chloroform/methanol (2:1, v/v) mixture followed by centrifugation to separate the phases. This extraction procedure is performed twice. The chloroform layer containing the total lipids is saved and evaporated to dryness on a rotary evaporator using nitrogen.

Next, the various lipid classes in the tissues will be separated by thin layer chromatography. Approximately 5 µl of a solution containing 1 mg/ml lipid is spotted onto silica gel TLC plates and eluted with petroleum ether/diethyl ether/acetic acid (80:20:1, v/v/v). After drying, the bands can be visualized under UV light (375 nm) by spraying with 0.1% 2,7-dichlorofluorscein. The appropriate classes will be identified by appropriate standards and scraped off into reaction vials.

Methylation of the component fatty acids will be performed by modification of the transesterification method of Morrisson et al. (38). Methanol (350 µl), benzene (300 µl) and 14% (w/v) boron trifluoride (350 µl) is added to the reaction vials containing the isolated lipids. The vials are sealed under nitrogen and heated in a closed steam bath for 45 min. After cooling 1 ml of water is added to each vial and the methyl esters are extracted with 2 ml of petroleum ether. The ether layer is dried and evaporated under a low flow of nitrogen. The solvent is evaporated to a volume of 100 µl. At this stage, the appropriate lipid classes have been isolated and their fatty acids methylated.

Methyl esters will be separated and quantitated by gas-liquid chromatography as described by Beynen and Katan (39) on a Hewlett Packard 5890 A gas chromatograph equipped with a flame ionization detector and a 1.8 m glass column (2 mm ID) filled with 10% Silar 5CP on 100-120 mesh chromosorb WHP (Supleco, Inc, Bellefonte, PA). Helium is used as the carrier gas at a flow rate of 5 ml/min. An injection volume of 1 µl, representing 2-4 µg of fatty acid, is required. The oven temperature is programed to increase from 120 °C in 20 min and remain constant at 215°C for 40 min, with the injector and detector temperatures set at 205 and 240°C respectively. Peaks are identified by comparison with authentic standards and quantified by area percentages.

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The fatty acid peaks of interest include: 18:3w3, 20:5w3, 22:5w3, 22:6w3, 18:2w6, 18:3w6, 20:3w6, 20:4w6, 16:0, 18:0, 16:1w7, and 18:1w9. The sums of all w6 and w3 families will be tabulated as well as the ratios of w3 to w6 and the polyunsaturated fatty acids (w3 and w6) to the saturated (16:0 and 18:0) fatty acids.

D 5.2) Lipid Analyses

Analyses of mouse plasma total cholesterol, high density lipoprotein and triglycerides will be performed using the Kodak Ektachem 400 Clinical Chemistry Analyzer. The analyzer employs a layered coating dry slide technology developed in 1983 (40,41).

D 5.3) Statistical Analysis of Expected Analyses

To determine whether tumor incidence varies as a direct function of dietary fat intake, differences in tumor incidences among the high fat, moderate fat and low fat groups will be compared using Bartholomews test for ordered alternatives (42). If the null hypothesis of no difference in tumor incidence among groups is rejected, pairwise comparisons will be conducted using the Fischer exact test with Bonferroni's adjustment to the a-value for multiple comparisons. The significance of differences in tumor multiplicity among the treatment groups will be assessed by 4 different methods. Differences in total tumor burden per group will be assessed for significance by the pairwise chi square test. Tests for trends will be conducted by Bartholomew's test (42). Multiplicity defined as the number of tumors per tumor bearing animal or the number of tumors per total number of animals at risk will be assessed by Student's t test; multiplicity assessed in terms of the frequency distribution of tumors per animal will be tested for significance by the chi test for homogenicity.

Total tumor volume will be determined by measuring each tumor in three dimensions using the formula for an ellipse ($4/3\pi R^2, R_2$) to calculate tumor volumes (34). Since tumor volume data will probably not be normally distributed, statistical comparisons will be conducted using the non-parametric Mann Whitney U Test. Animal weight gains will be analyzed using Analysis of Variance with repeated measures (43).

Since this is original research, no pilot study data are available for the determination of the sample size needed to assure a given power. The table below (Table 2) has been prepared to illustrate sample sizes needed for a variety of situations. Although there are basic differences between regression and correlation, a set of data for (Ho:β=o) would also yield a statistically significant correlation co-efficient (Ho:rho=o). Thus, sample size has been estimated by reference to the correlation coefficient (44,45). In order to detect a statistically significant correlation of 0.5 or better with p <0.05, then 50 animals would be required. Each study population will also be composed of an additional 30 mice that will not undergo treatment to act as sentinels.

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Table 2

Sample Size Needed for Various Values of Power and Various Correlation Coefficients in Testing HO:RHO=O

Correlation Coefficients	Power (1 - β) .75	.90	.95
0.3	75	113	139
0.5	26	38	46
0.7	12	17	20
0.9	6	8	6

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Proposed Budget

Personnel	Year 1 Effort		Year 2 Effort	
Daniel W. Sepkovic, Ph.D, Principal Investigator	30%	R	30%	
Nancy J. Haley, Ph.D., Consultant	---		---	
Ellen T. Louis, B.S., Chemist	50%		50%	R
Animal Biologist	516 hr		228 hr	
Animal Costs [a]				
Purchase		2,640		---
Maintenance		9,673		4,600
Diet (AIN-76A)		6,470		2,400
Supplies		750		750
Chemicals				
GC (900 samples: 450 lung tissue and 450 lung tumor)		2,500		2,500
lipid analyses (cholesterol, HDL and triglyceride analyses of 450 samples)		2,000		2,000
Pathology [b]		9,000		2,700
Safety supplies for nitrosamine work (NNK)		3,000		---
Miscellaneous		800		800
Total direct costs		72,702.		51,160.
69% Indirect costs		50,164.		35,300.
Total		122,866.		86,460.

[a]) Animal maintenance and diets in Year 1 for twenty week study (300 mice) and one half of the twenty month study. Year 2 is for completion of the twenty month study.

[b]) Pathology in Year 1 - 300 animals at 5 slides/animal times $6.00/slide. In Year 2 - 90 animals at the same rate.

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R: REDACTED MATERIAL

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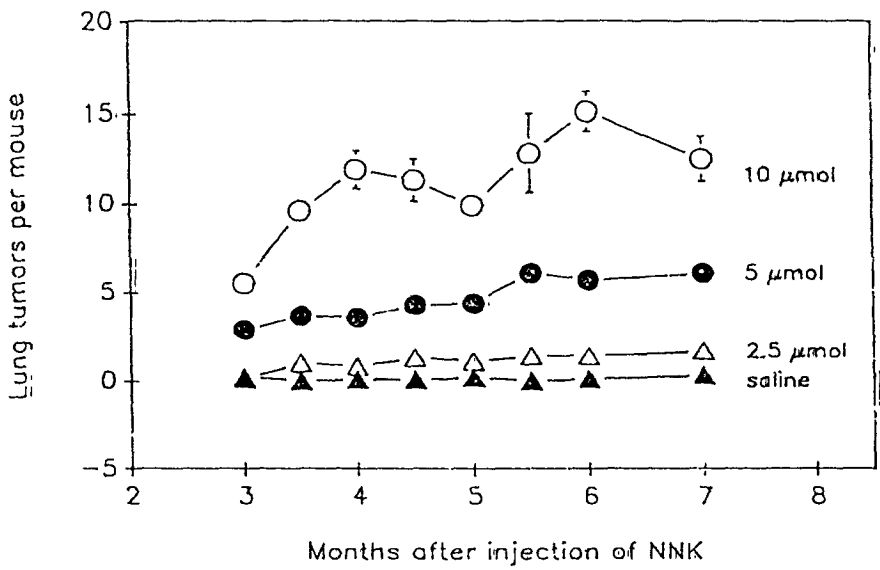
FIGURE 1

LUNG TUMOR INCIDENCE IN A/J MICE TREATED WITH NNK



After Hecht et al. (32).

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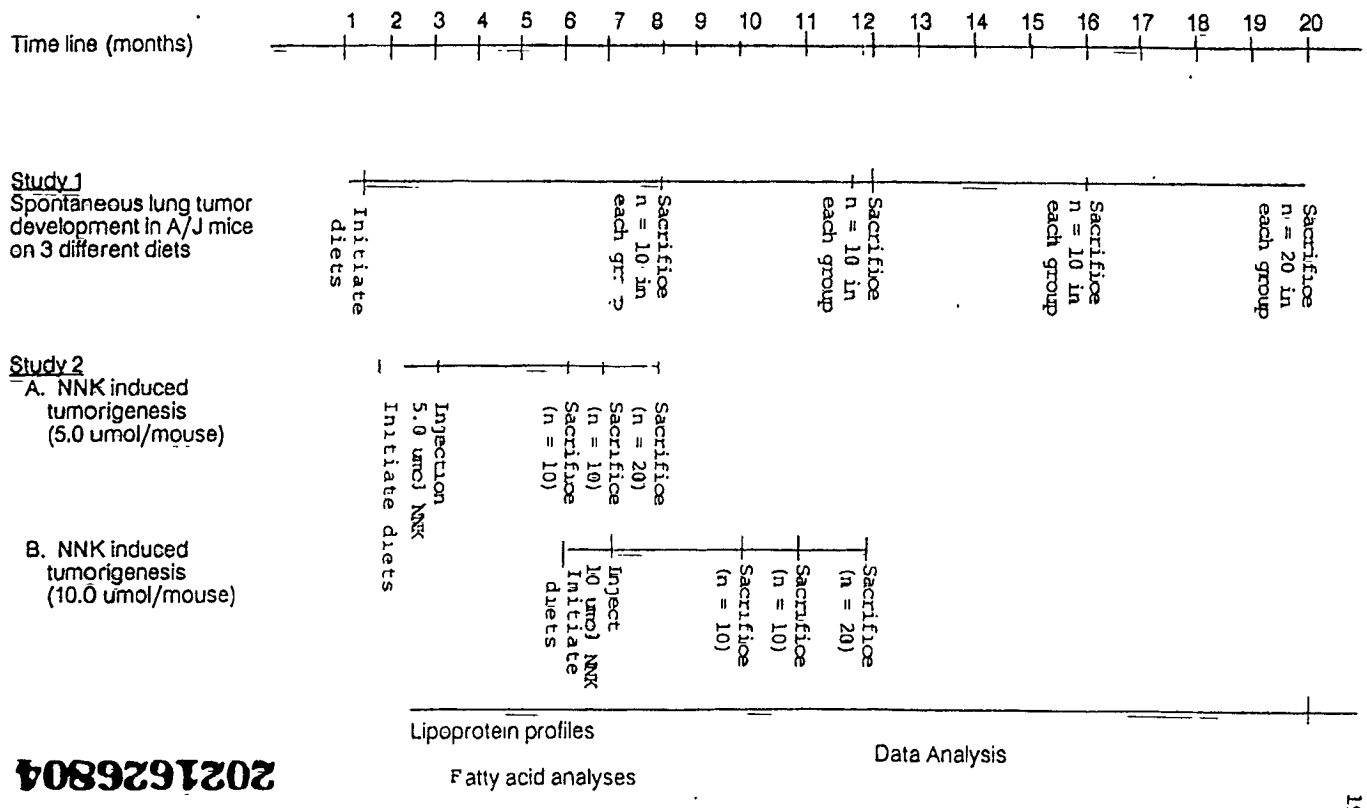
Proposed Study Time Course



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DANIEL W. SEPKOVIC, Ph.D.

Education

Western Connecticut State College, Danbury, CT - 1976. B.A. Biology/Chemistry

Fordham University, Bronx, NY - 1978. M.S. Biology (Cytology)

Fordham University, Bronx, NY - 1982. Ph.D. Endocrinol/Toxicol.

American Health Foundation, Valhalla, NY - 1982. Post-Doctoral

Professional Experience

1985-present	Senior Associate, Section of Clinical Biochemistry, Division of Nutrition and Endocrinology, American Health Foundation, Valhalla, NY.
1984-1985	Associate, Section of Clinical Biochemistry, Division of Nutrition and Endocrinology, American Health Foundation, Valhalla, NY.
1983-1985	Adjunct Assistant Professor of Biology, Bronx Community College, Bronx, NY.
1982-1984	Post-Doctoral Fellow at the American Health Foundation. Valhalla, NY. Areas of investigation focus on nicotine toxicology and the health effects of tobacco smoke components, in the biochemical effects of tobacco smoke metabolites and in the hormonal perturbations caused by the use of tobacco products. Special emphasis is placed on the relationship between cigarette smoking, low thyroid function and atherosclerosis.
1980-1981	Teaching Fellow at Fordham University supervising undergraduate Zoology and General Biology laboratories.
1980-1982	Research Assistant-Specialization in endocrine physiology and the effects of environmental toxicants on hormonal regulatory mechanisms. Thesis title: Effects of PCB and PBB Isomer on Triiodothyronine Secretion and Degradation, Adrenal Function and Cationic Transport.
1979	W. Alton Jones Foundation Fellowship for the study of vertebrate cell and organ culture at Lake Placid, NY.
1979-1980	Instructor of Biology, Mount Sinai School of Medicine SETH Program. Responsibilities included course development and instruction in Anatomy and Physiology, Cell Biology, Health Career Science, Genetics and General Chemistry.
1978-1979	Instructor of Biology at the College of Mount Saint Vincent, Riverdale, NY. Duties included teaching and supervision of General Biology laboratories.
1976-1979	Graduate Assistantship at Fordham University. Responsibilities included supervision of Electron Microscopy, Microbiology and Zoology laboratories. Duties in electron microscopy involved the supervision of graduate students in TEM techniques specifically, fixation embedding and ultrastructural examination of mammalian and isopod tissue.

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Haley, N.J., Sepkovic, D.W., Louis, E. and Hoffmann, D. Absorption and elimination of nicotine by smokers, nonsmokers and chewers of nicotine gum. In: The Pharmacology of Nicotine, (M.J. Rand and K. Thrau, eds.), Vol. 9, IRL Press, Washington, D.C., 1987, pp. 20-22

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NANCY JEAN HALEY, PH.D.

Education

St. Joseph's College, Brooklyn, NY - 1970. B.A. Biology/Chemistry

St. John's University, Jamaica, NY - 1972. M.S. - Biochemistry

St.. John's University, Jamaica, NY - 1975. Ph.D. - Biochemistry

Rockefeller University, New York, NY - 1975-1978 Post-Doctoral - Atherosclerosis

Professional Experience

1985-present	Associate Chief, Division of Nutrition and Endocrinology, American Health Foundation, Valhalla, NY.
1984-1985	Head, Section of Clinical Biochemistry, Division of Nutrition and Endocrinology, American Health Foundation, Valhalla, NY.
1981-1984	Associate Research Scientist, Division of Nutrition and Endocrinology, American Health Foundation, Valhalla, NY.
1980-1981	Research Scientist, Division of Nutrition, American Health Foundation, Valhalla, NY.
1977-1980	Research Associate, Laboratory of Biochemical Cytology, The Rockefeller University, NY.
1975-1977	Post-Doctoral Fellow, Laboratory of Biochemical Cytology, The Rockefeller University, NY
1972-1975	Clinical Technologist, Clarendon Laboratories, Brooklyn, NY.
1970-1975	Graduate Research Fellow, Department of Biological Science, St. John's University, Jamaica, NY

Awards and Commendations

1969	National Science Foundation Research Scholarship, Brown University, Providence, RI
1972	National Defense Research Fellowship
1973	Teaching Fellowship, St John's University
1975	Research Award, U.S. Public Health Service (GM07245 01)
1977	Research Fellowship, National Heart, Lung and Blood Institute (1F32 HL05516 01)
1985	Traveling Fellowship, American Heart Association

Certifications

Department of Health, City of New York, Clinical Laboratory Supervisor R 233905.
Department of Health, City of New York, Clinical Laboratory Technologist R 232262.
Department of Health, State of New York, Clinical Laboratory Director (pending, 1989).

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Etzel, R.A., Greenberg, R.A., Haley, N.J. and Loda, F.A.
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Mattson, M.E., Boyd, G., Byar, D., Brown, C., Callahan, J.F., Corle, D., Cullen, J.W., Greenblatt, J., Haley, N.J., Hammond, S.K., Lewtas, J. and Reeves, Warren.
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Effect of low levels of smoking on estradiol metabolism in women. Lancet (In press).

Bauman, K.E., Koch, G.G., Bryan, E.S., Haley, N.J., Downton, M.I. and Orlandi, M.A.
On the measurement of tobacco use by adolescents: Validity of self-reports of smokeless tobacco use and validity of cotinine as an indicator of cigarette smoking. Am. J. Epidemiol. 1989 (In press).

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INSTITUTIONAL ENVIRONMENT AND RESOURCES

The American Health Foundation's physical facilities are located at two sites to permit maximum effectiveness in the diverse research activities undertaken. The Naylor Dana Institute for Disease Prevention, opened in 1975 on the Westchester County Medical Center reservation in Valhalla, New York, is the primary laboratory research facility of the Foundation. The Mahoney Institute for Health Maintenance is located in the Ford Foundation building, at 320 East 43rd Street, New York City, to facilitate the study of population by the Foundation's Division of Epidemiology, for clinical trial studies, and for risk factor reduction research and demonstration programs. The Foundation attempts to provide surroundings most conducive to the highest quality research.

The Naylor Dana Institute for Disease Prevention, houses the specialized laboratory facilities for the Divisions of Environmental Carcinogenesis, Chemical Carcinogenesis, Nutrition and Endocrinology, Pathology and Toxicology and Molecular Biology and Pharmacology. The building includes modern facilities for instrumental analysis, synthesis of radioactive and carcinogenic chemicals, tissue culture, clinical chemistry, pathology, histology, and microbiology. A detailed description of facilities is given below. Institute administrative areas include offices for the President, Vice-President for Research, Institute Administrator, and other line management personnel, library and reference services, staff dining facilities, and maintenance areas. The building is adjacent to the New York Medical College Basic Sciences Building, where American Health Foundation principal staff have Research Professor appointments.

Tobacco and Health Research Studies at AHF

Studies on tobacco and health at the American Health Foundation are conducted with an interdisciplinary research team and include epidemiologic investigation, behavior modification, health education, chemistry and biochemistry, biology, pathology and clinical chemistry. The overall program is directed by Ernst L. Wynder, M.D. (President, American Health Foundation, Epidemiology Division) in collaboration with Stephen S. Hecht, Ph.D., Director of Research; Gary M. Williams, M.D., Director of Medical Sciences; Dietrich Hoffmann, Ph.D., Associate Director and Chief, Division of Environmental Carcinogenesis (chemistry, biochemistry, environmental carcinogenesis), David P. Rose, M.D., Ph.D., Sc.D., Associate Director and Chief, Division of Nutrition and Endocrinology; Abraham S. Rivenson, M.D., Chief, Histopathology Facility and Nancy J. Haley, Ph.D., Associate Chief, Division of Nutrition and Endocrinology and Head, Section of Clinical Biochemistry.

Available instrumentation in the Divisions of Environmental Carcinogenesis and Chemical Carcinogenesis include the following:

1. 60 MHz Perkin Elmer high resolution NMR Spectrometer.
2. 90 MHz Jeol Model FX90Q High resolution Fourier Transformation Multi-Nuclear magnetic Resonance Spectrometer System.
3. Perkin Elmer Model 21 infrared spectrophotometer.
4. Perkin Elmer Model 267 infrared spectrophotometer.
5. Cary Model 118 UV spectrometer.

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6. Hewlett Packard Model 5981A dual source combined gas-chromatography-mass spectrometer-computer system.
7. Three Waters Associates Model 204 high pressure liquid chromatographs complete with solvent programmers and UV detectors.
8. Dupont Model 820 high pressure liquid chromatograph system.
9. Waters Associates preparative high pressure liquid chromatograph system 500.
10. Thermal Electron thermal energy analyzer equipped with a Hewlett Packard Model 720 gas chromatograph and Waters Associates high pressure liquid chromatographic Model 6000A pump for nitrosamine specific detection.
11. Three Hewlett Packard Model 7620A gas chromatographs equipped with thermal conductivity, flame ionization, and electron capture detectors.
12. Six Hewlett Packard Model 5710A gas chromatographs equipped with flame ionization detectors.
13. Hewlett Packard Model 5830 chromatograph for capillary GC with flame ionization and electron capture detectors.
14. Hewlett Packard Dual N-P gas chromatograph with FID control for nitrogen specific compound detection.
15. Hewlett Packard Model 894 gas proportional counter for ^{14}C specific detection.
16. Nuclear Chicago scintillation counter Model Isocap 300.
17. Perkin Elmer Model 460 atomic absorption spectrometer.
18. New Brunswick Scientific Model B64 freeze-dryer.
19. Varian Model 430020 fluorescence detector for high pressure liquid chromatography.
20. Beckmann Model 25 UV spectrophotometer for variable UV scanning with High pressure liquid chromatography.
21. Aminco DW2AS UV-VIS scanning spectrophotometer.
22. Aminco-Bowman scanning spectrofluorometer.
23. Gilford 2400-S recording spectrophotometer.
24. Sorvall RC2B refrigerated high-speed centrifuge.
25. Two Dubnoff metabolic shaking incubators.
26. Brinkmann Polytron.
27. Spinco Model L preparative ultra-centrifuge.
28. Gilson GME oxygraph.
29. Thermal Energy Analyzer with GC Model 543
30. $NO/NO_2/NO_x$-Analyzer, Model 1600, CSI

Because of our long experience in tobacco research and indoor pollution, we have an extensive literature collection on the subjects and a depository of several hundred pure constituents of tobacco and tobacco smoke.

The Division of Environmental Carcinogenesis has an Analytical Smoking Laboratory and a laboratory in the basement of the Institute for the preparation of large volumes of tobacco "tars" (capacity ≈1 kg "tar" per week). In addition to the Phipps and Bird 20 channel-FTC

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smoking machine, we have smoking devices for the smoking of single, 4, 20 and 30 cigarettes, primarily "Borgwaldt" instruments. For the large scale "tar" preparation we employ 4-30 channel individual smokers. The Research Animal Facility contains a smoking room with 4 Hamburg Smoke Inhalation Devices, each has holding space for the concurrent smoke exposure of 10 Syrian golden hamsters.

Clinical Biochemistry, Division of Nutrition and Endocrinology

The requirements for this proposal will be carried out in three well-equipped laboratories in the Section of Clinical Biochemistry at the Naylor Dana Institute.

Equipment in these laboratories necessary to this proposal include an IL-282 CO-Oximeter, two International centrifuges for blood separation, two complete trains of Auto Analyzer (Technicon) components including scintillation counter and automated pipettors and dilutors necessary for radioimmunoassay. In addition, the laboratories contain a Beckman Airfuge ultracentrifuge, an Eppendorf microfuge system, an atomic absorption Spectrophotometer, Perkin Elmer Model 460, a Gilford Computer directed Autoanalyzer Model 3500 and a Digital Instrument Computer VT 180 complete with data base and statistical software.

Other available instrumentation to the Division of Nutrition and Endocrinology are listed below:

1. Hewlett Packard 5890A Gas Chromatograph
2. Variable Wavelength Kratos Detector Model SF-769P
3. Revco Freezer
4. Hewlett Packard 7673A Controller
5. Waters Associates Solvent Programmer
6. Waters Associates Chromatography pump
7. Waters Associates Absorbance Detector
8. Gilford Spectrophotometer 2400S
9. Mettler Scales H 31AR and P 1210
10. Damon/IEG Centrifuge
11. Spinco Ultracentrifuge
12. ICR Centrifuge
13. CO_2 Incubator (New Brunswick)
14. Searle X-Ray Spectrometer Model 1186
15. Micromedic Systems Autopipettors
16. Instrumentation Labs CO-oximeter IL-282
17. Dynatech MR700 ELISA Reader
18. Atomic Absorption Spectrophotometer Model 460
19. Micromedic Reagent Dispenser
20. Technicon A.A. Sampler, Colorimeter, Pump Manifold and Recording System
21. Gilford Computer and Autoanalyzer 3500
22. TM Analytical 1191 Gamma Counting System
23. Beckman LS 6800 Scintillation Counter
24. Sorvall QTD65B Ultracentrifuge
25. Waters Model 510 HPLC Pump
26. Waters Data Module

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27. BAS LC-4B Amperometric Detector (two)
28. Instrumentation Laboratories CO-oximeter 282
29. Digital VT 180 Computer with 4 disc drives
30. Digital Decuriter IV
31. Digital DFO3 Modem
32. Beckman Airfuge
33. Gilford Stasor III Spectrophotometer

For dosimetry in uptake of smoke components from polluted indoor environments, we utilize a $16m^3$ enclosed room wth 6 air exchanges per minute which is devoid of upholstered furniture or drapery. Sidestream smoke is generated by uninterrupted machine smoking of 28 cigarettes during 80 minutes. Smoke particulates, nicotine, carbon monoxide and hydrogen cyanide concentrations in the ambient air are monitored.

In addition, the Division of Pathology and Toxicology includes two modern cell and tissue culture facilities. These laboratories are equipped with 3 laminar airflow tissue culture hoods, 3 Baker class II NCB-4 hoods hooked onto exhaust systems which are exclusively used for the experiments involving carcinogens and radioisotopes, a walk-in incubator, a walk-in cold room, a ZBI Coulter Counter, a Model 880 Artek Counter with a Model 700 Computer-Print Printer, two phase-contrast microscopes with photomicroscopy attachments, 5 environmentally controlled cell culture incubators and other basic equipment for cell and tissue culture work. For safe weighing of chemicals, the Division has a Mettler H20T analytical balance in a Germ Free Lab Biological Safety glove box. Additionally, the Division has access to an ISCD Density Gradient Fractionator with a UA-5 Absorbance Monitor, a Beckman L5-75 ultracentrifuge and a Beckman LS-3133P liquid scintillation counter.

Histology Facility

The Histology Facility of the American Health Foundation processes tissues, cell suspensions or smears for microscopic and ultra-microscopic examination as per the requests by individual investigators from the various divisions of the American Health Foundation. The majority of samples are submitted by the Research Animal Facility.

The histology laboratory is the largest single laboratory in the Division of Pathology and Toxicology. It includes a main processing room, pathologist's office, dark room and storage space (950 sq. ft.).

The personnel, organization, procedures and equipment are similar to their counterparts in histopathology laboratories in hospitals. The personnel in the histology facilities consists of one pathologist director and four histology technicians. The director is Dr. Abraham Rivenson, M.D., certified in Anatomic Pathology. He supervises the conduct of the entire process, sets technical and administrative priorities, evaluates the quality of the routine and histochemical strains, coordinates activities with Dr. G. Williams, the Chief of the Division, and with other pathologists and investigators in determining the specific histopathology needs of each particular experiment or study.

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AAALAC and is an institutional member of AALAS. The facility is inspected twice a year by both Federal and State governments, and has repeatedly had high ratings on such inspections.

Biosafety and Biohazard

The Biosafety and Biohazard Facility, supported by a Cancer Center Support Grant, is responsible for formulation ad implementation of safety rules and regulations throughout the entire institute. The Biosafety and Biohazard Office consists of the Safety Officer and Safety Committee.

The Safety Officer, Ms. Laura Rozycki, holds a full-time position. She ascertains that all laboratory and operational practices and procedures comply with Federal and State laws. She updates and recommends to the Senior Staff improvements in safety policy regarding all operations conducted at the American Health Foundation.

The Safety Officer provides a variety of safety related functions at the Foundation. She maintains an on-going program of medical monitoring for each employee. This includes an annual physical exam wich is scheduled by the Safety Officer. Prior to the physical exam, blood work and medical history data are collated and submitted to the physician. The Safety Officer investigates and submits reports on injuries, fires and other accidents and periodically inspects laboratories and operational areas periodically. She supervisors the purchase of safety-related equipment and disposal of hazardous materials. One of her most significant functions is to monitor working areas necessary to assure a safe research environment. The Safety Officer orients all scientific staff in proper safety procedures before work in the laboratory can commence.

The Safety Committee consists of representatives from each division or section who are selected by the Safety Officer with the approval of the appropriate supervisor. Members of the Safety Committee act as liaisons between the Safety Office, and their co-workers. Their responsibility is to observe, evaluate and report hazards before they cause accidents.

The Safety Officer is also the Radiation Safety Officer, subject to approval by the New York State Department of Health. She maintains the license granted by New York State to the American Health Foundation to use radioisotopes for research purposes. This includes conducting a bi-monthly inventory of radioisotope stores within the Foundation and supervising the proper disposal of radioactive wastes. The Radiation Safety Officer serves a chairman of the Radiation Safety Committee, which is composed of representatives from each division in which radioisotopes are handled. This committee sets and enforces policies concerning the safe handling of radioisotopes at the American Health Foundation.

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Data Management and Computing Facilities

The Biostatistics and Computer Services Facility provides the data management and processing capability for analysis of large cross-sectional and prospective data bases.

The facility is currently staffed by a senior Biostatistician, Programmers, Systems Analyst/Programmers, Applications Programmers, Data Coders, and Secretary/Editor. Acting Director of the Division is Dr. James R. Hebert.

The primary functions of the Facility are:

- to collaborate with AHF researchers in all phases of research, including initial planning, study design, data collection and analysis interpretation of results, and preparation of manuscripts.

- to provide the data management skills to ensure that the data generated by the research projects are managed and processed as efficiently as possible.

- to perform the data processing and computing services required by the researchers.

The main computer is a Digital Equipment VAX 11/750 with two disc drives running the VMS operating system. The system has Fortran and PLI compilers and plans are underway to purchase a C compiler. Statistical analyses are done on the Statistical Analysis System (SAS) and SPSS.

The VAX computer is hardwired to most offices of professional staff at the New York site. Plans are underway to install a terminal and modem in Valhalla to tie in with the NYC equipment. The computer is also provided with communications software and hardware so as to make most efficient use of the 25 microcomputers located in New York City, the Naylor Dana Institute in Valhalla, N.Y. and those located in the field.

Computer Facility in Clinical Biochemistry

The Clinical Biochemistry Section has a dedicated IBM System 36 Computer which is used for maintenance of participant records and research studies. The system functions on a laboratory accession number method and maintains quality control across analyses by date, method and staff member. Additional computer support is available through 3 laboratory microcomputers which also emulate System 36 terminals. Two of these microsystems collect instrument data directly from liquid scintillation counters and the Kodak 400 analyzer and direct data into the appropriate accession records. This keeps data entry errors to an absolute minimum. Entries are checked daily by terminal scanning and a Systems Operation Manager monitors the entries each week with checks for accuracy and cross reference.

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